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THE HISTOGENESIS AND CYCLIC PHENOMENA OF THE SINUS GLAND AND X–ORGAN IN CRUSTACEA

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Introduction

In the decade that has followed Hanström's (1933, 1934) description of the sinus gland and X-organ in Crustacea a number of investigators, Sjögren (1934), Hanström (1937), and Ståhl (1938) have described them in detail. All of these studies have been concerned with a description of these glands as they appear in the adult animal. Since there has been little or no work done upon the histogenesis of either the sinus gland or X-organ, it is one of the objects of this paper to describe the histogenesis of both the sinus gland and X-organ in detail.

The endocrine activity of the sinus gland has been more or less well established through numerous studies in the past several years. As these are quite adequately and critically examined by Scharrer (1941) and Kleinholz (1942) there is no need to review the literature in detail. For further information of this nature one should examine those papers. Although extensive physiological studies have been made in relation to the endocrine function of the sinus gland, there have been no cytological studies made (except in Cambarus by Dethier 1942) to determine whether or not there are any evidences of cyclic phenomena in this gland. The role of the X-organ has been suggested, but no cytological studies have been made of it. Both the X-organ and sinus gland have been cytologically examined and the results of this study are reported herein.

Methods and Materials

The histogenesis of the sinus gland and X-organ were studied in two species of Crustacea, *Homarus americanus* and *Pinnotheres maculatus*. The adults of these species and of *Cambarus virilis* were studied for cytological evidences of cyclic phenomena during the moulting period.

The eggs of Homarus were fixed in Carnoy-Lebrun: the first four stages after hatching were fixed in Zenker-formol and Bouin-Duboscq-Brasil, and the adult eye stalks (one week, 48 hours, six hours before, during, six hours, 48 hours, one,

[1] Contribution No. 326 from Woods Hole Oceanographic Institution.

one and one-half, four, six and thirteen months after moulting)[2] were fixed in Zenker, Zenker-formol and Bouin-Duboscq-Brasil. The eggs and first four stages after hatching were doubly imbedded in parlodion and paraffin and sectioned at five to nine micra. In some of the adults the exoskeleton of the eye stalk was decalcified and the whole eye stalk was doubly imbedded in parlodion and paraffin and sectioned at seven to 12 micra. In other adults the exoskeleton of the eye stalk was removed and the specimens were singly imbedded in paraffin. These were sectioned at seven to 15 micra.

The eggs, first zoea and adult stages (before, during, after and between moulting periods) of Pinnotheres were studied. The eggs and first zoea were fixed in Carnoy-Lebrun, Zenker-formol and Bouin-Duboscq-Brasil, and were doubly imbedded in parlodion and paraffin. Sections were cut at four to seven micra. The various stages of the adult were fixed in Zenker-formol and Bouin-Duboscq-Brasil, and were doubly and singly imbedded and sectioned at six to 12 micra.

The eye stalks of Cambarus were treated in the same way as those of Homarus; some were singly and some doubly imbedded. Sections were cut at seven and nine micra.

Serial sections were made of all specimens and these were stained with haemalum and eosin, Mallory's triple, Foot's (1933) and Lillie's modifications of the Masson trichrome stain, and the protargol method of Bodian (1937).

Observations

A. *Pinnotheres maculatus*

The X-organ is found in the embryo just before hatching (Figs. 1, 19) in that part of the eye which will become the median ventral side of the eye stalk in the

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Plate I[3]

The histogenesis of the sinus gland and the X-organ in *Pinnotheres maculatus*. All figures are oblique frontal sections of the right eye stalk. The neuropile of the optic ganglion is white and the ganglion cell layer is stippled.

Figure 1. Section of the late egg stage showing the position of the X-organ in relation to the structures of the optic ganglion. X—X-organ. S. G.—Sinus gland.

Figure 2. Section of the first adult stage showing the positions of the sinus gland and X-organ: both are distal to the medulla terminalis.

Figure 3. Section of the second adult stage. The sinus gland has begun to move distally, but the X-organ is found in the same general position as in earlier stages.

Figure 4. Section of the third adult stage. The sinus gland has advanced to a point between the medulla interna and medulla externa.

Figure 5. Section of the fourth adult stage. The sinus gland is lateral to the medulla externa at this stage.

Figure 6. Section of the fifth adult stage. The sinus gland now occupies a position between the medulla externa and lamina ganglionaris.

Figure 7. Section of the sixth adult stage. The sinus gland has advanced to a point that is distal to the lamina ganglionaris. In this as in previous stages the X-organ is found distal to the medulla terminalis.

[2] I am indebted to Dr. Charles J. Fish and the staff at the Wickford Hatcheries for the determination of the times in those specimens that were about to moult.

[3] All figures have been drawn with the aid of a micro-projection apparatus. All structures found between the hypodermis of the exoskeleton and the optic ganglia have been omitted for the sake of clarity.

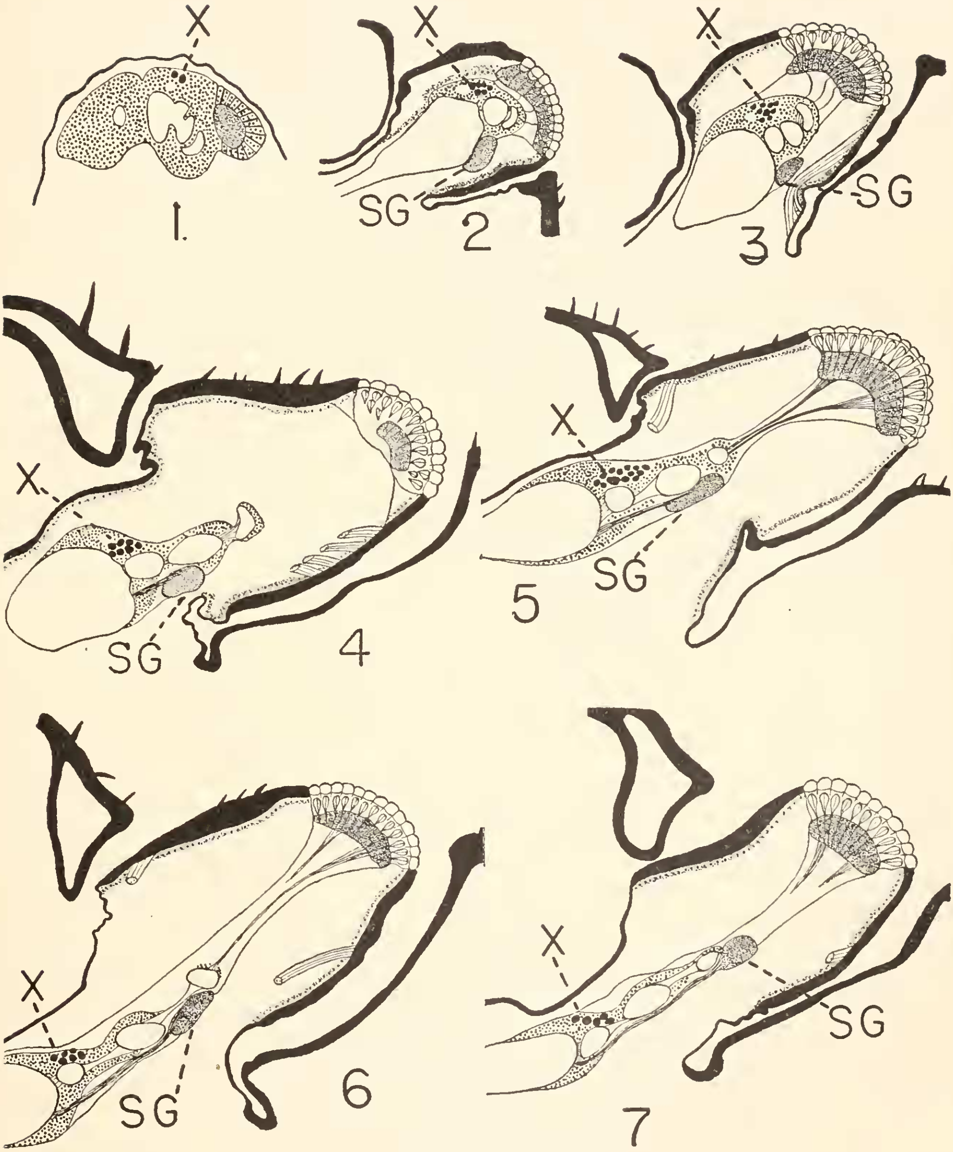

PLATE I

first zoea. It occupies a position on the distal portion of the medulla terminalis and is almost entirely surrounded by the cells of the optic ganglia. There is also found, usually between the X-organ and the medulla terminalis, an area which is devoid of cells; this appears in serial sections as a hole. As far as can be determined from cytological preparations the cells of the X-organ are very similar to those found in the ganglion cell layer. They are probably derived from the same embryonic source and later become differentiated into X-organ cells. There is no evidence of any nerve fiber connections with the medulla terminalis. The nuclei are of the same size, shape and appearance as the nuclei of the ganglia cells. There is more cytoplasm present than in the ganglia cells; it is non-granular and clear. The X-organ is an integral part of the ganglia cell layer and is not set apart from it by a connective tissue sheath.

The secretory products of the X-organ are large rounded masses which exhibit concentric rings; this seems to indicate that the secretions have been laid down at different intervals. These secretory products always give a basophilic reaction when stained; they are blue after aniline blue and are structurally very similar to those found in the X-organ of Homarus. There is no evidence of any cyclic phenomena in the egg stage as the secretions have the same characteristic appearance in all specimens.

There is no evidence that the sinus gland has been developed by the late egg stage or the first zoeal stage. Unfortunately, conditions existing at Woods Hole last summer did not permit obtaining the intermediate forms between the first zoeal and the first adult stage so that these could not be studied. Attempts to raise them beyond the first zoeal stage were fruitless.

The adults may be grouped into six categories or stages which correspond to the moults. This is comparable to the five moults found in *Pinnotheres pisum* by Atkins (1926). In all stages of the adult (Figs. 2–7, 21) the X-organ is found in the same relative position that it occupies in the egg stage. The number of cells of which it is composed increases after each moult, but no mitoses were observed at any time. The cells are larger than the ganglia cells which surround them. They are wedge-shaped and are grouped in such a way as to remind one (when examining serial sections) of a pie that has been cut; the nuclei are found around the periphery and each cell becomes narrower as its cytoplasm extends toward the center of the X-organ. As the cytoplasm becomes filled with the secretory products the nucleus is pushed more and more toward the periphery

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PLATE II [4]

The histogenesis of the sinus gland and X-organ in *Homarus americanus*. All figures are oblique frontal sections; figures 8, 9, and 11 are of the left eye stalk, and figure 10 is of the right eye stalk. The neuropile of the optic ganglion is white and the ganglion cell layer is stippled.

FIGURE 8. Section of the late egg stage showing the position of the X-organ.

FIGURE 9. Section of the third stage after hatching. The sinus gland is seen as a thin structure on the proximal side of the medulla externa.

FIGURE 10. Section of the fourth stage after hatching. The sinus gland still occupies a position on the proximal side of the medulla externa. The X-organ extends to the hypodermis of the exoskeleton where the eye papilla is found.

FIGURE 11. Section of the adult stage. Both the X-organ and sinus gland, on opposite sides of the eye stalk, are seen extending beyond the limits of the neurilemma which surrounds the optic ganglion.

[4] See footnote 3.

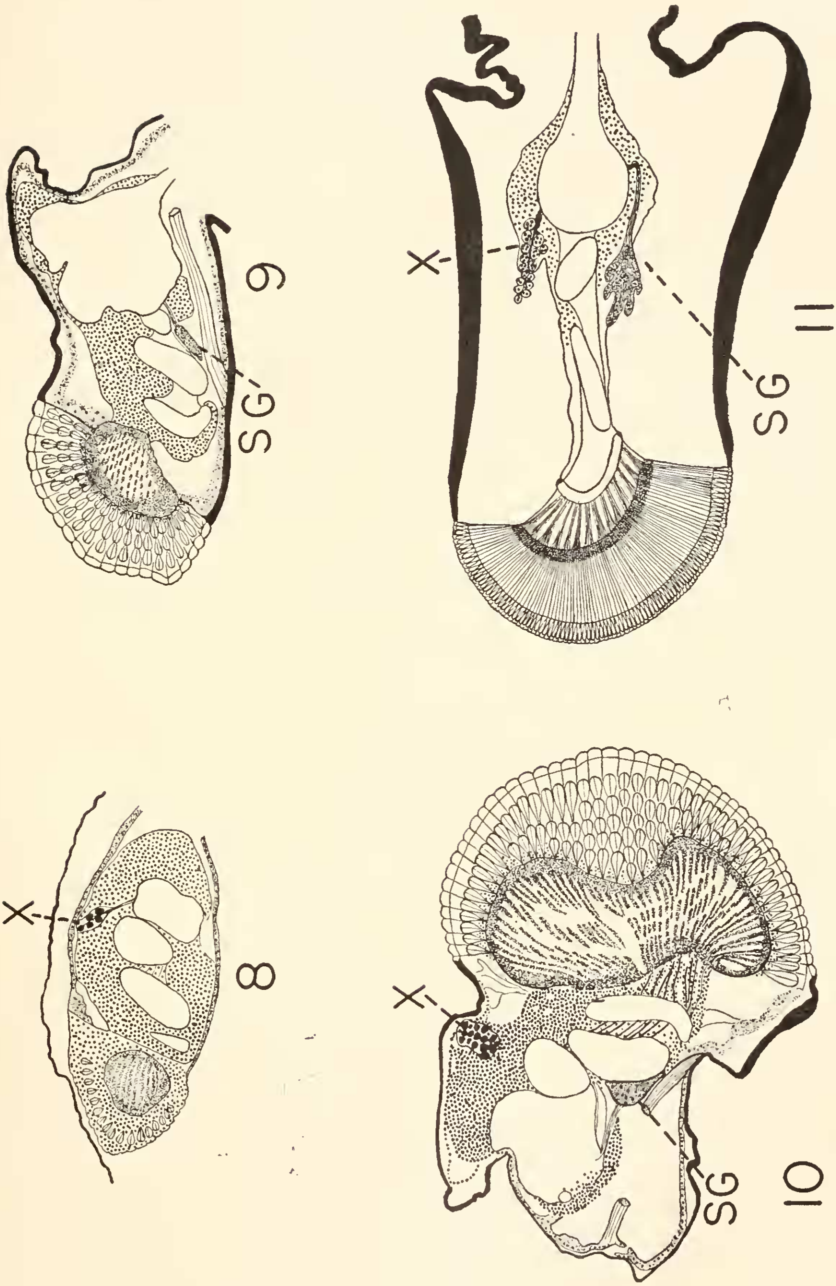

PLATE II

of the cell. In no stage is there any evidence that the X-organ has a nerve fiber tract which extends from it to the medulla terminalis. Cytologically there is no evidence of any cyclic phenomena associated with the secretion processes of the X-organ at any time. It always exhibits the same basophilic reaction regardless of whether it is fixed before, during, after or between moulting periods.

The sinus gland is well developed by the time the animal reaches the first adult stage (Fig. 2). It is at this time that it enters the mussel, *Mytilus edulis*, and begins its parasitic form of existence. The most remarkable feature in the subsequent development of the sinus gland is its change of position in relation to the structures of the optic ganglia. In the first stage (Fig. 2) it is found closely appressed to the medulla terminalis; in the second stage (Fig. 3) it has begun to move away from the medulla terminalis. In each successive stage it advances farther toward the distal portion of the eye stalk. In the third stage (Fig. 4) it occupies a position between the medulla interna and the medulla externa, in the fourth stage (Fig. 5) alongside the medulla externa, in the fifth stage (Fig. 6, 21) between the medulla externa and lamina ganglionaris, and in the sixth stage (Fig. 7) it has advanced to a point that is distal to the lamina ganglionaris.

In all stages the sinus gland is found on the dorso-lateral side of the eye stalk. There are very few nuclei in it (Fig. 23) and these bear such a close resemblance to those of the neurilemma, which is continuous with the sinus gland, that one might well consider the sinus gland a modification of the neurilemma (cf.

Plate III[5]

Microphotographs to show the cyclic phenomena in the sinus gland of *Homarus americanus* and *Cambarus virilis*. All photographs are × 725 reduced about 35 percent, and are of materials fixed in Bouin-Duboscq-Brasil and stained with Foot's modification of the Masson trichrome stain.

Figure 12. *Homarus americanus*. A portion of the edge of one lobule of the sinus gland of a specimen fixed forty eight hours before moulting and sectioned at twelve micra. The brilliantly staining acidophilic secretory products are seen as dark irregularly shaped masses. This and succeeding figures show the loose network of connective tissue which constitutes the framework of the gland and the very few scattered nuclei.

Figure 13. *Homarus americanus*. A portion of the edge of one lobule of the sinus gland of a specimen fixed six hours after moulting and sectioned at ten micra. The bulk of the secretory masses are only slightly acidophilic and appear less dark in the photograph.

Figure 14. *Homarus americanus*. A portion of the edge of one lobule of the sinus gland of a specimen fixed six months after moulting and sectioned at twelve micra. The secretory material is reduced in quantity and stains in a slightly acidophilic manner.

Figure 15. *Cambarus virilis*. A portion of the sinus gland of a specimen fixed before moulting and sectioned at nine micra. The numerous brilliantly acidophilic secretory masses are seen as dark masses hung upon the connective tissue framework of the gland. The blood sinus shows as clear areas.

Figure 16. *Cambarus virilis*. A portion of the sinus gland of a specimen fixed after moulting and sectioned at nine micra. The secretory products are conspicuous by their absence. The blood sinuses are filled with blood. The nuclei are scattered at random in the loosely arranged connective tissue.

Figure 17. *Cambarus virilis*. A portion of the sinus gland of a specimen fixed in December and sectioned at nine micra. The majority of the secretory products present are acidophilic. The blood sinuses appear as clear areas.

[5] All microphotographs were made using Bausch and Lomb microphotographic equipment. The photographs were taken on Eastman Super Panchro Press film, and were printed on Eastman Azo F-2, and Velour Black S-4 paper. Wratten filters G No. 15 and X-1 were used.

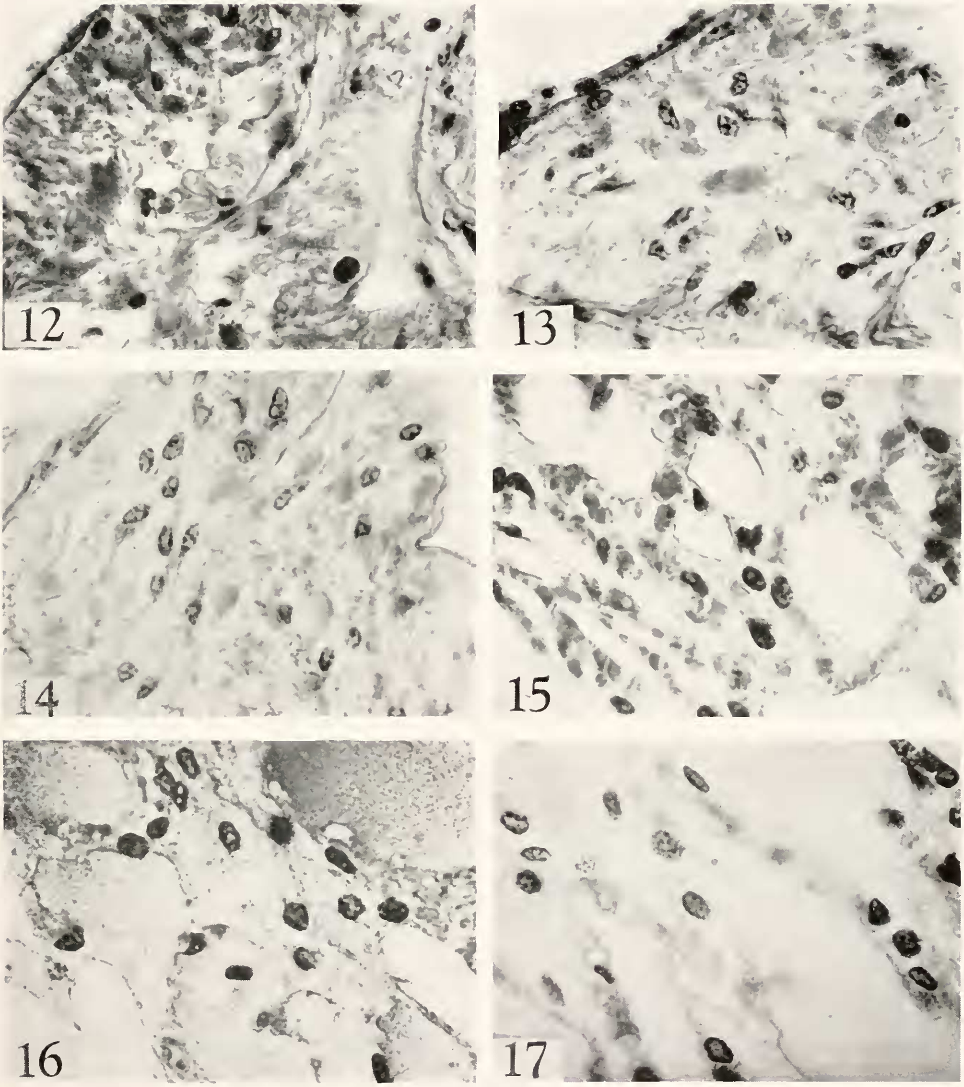

PLATE III

Hanström, 1939). In no stage of the adult is the sinus gland more than partially extruded beyond the level of the neurilemma. In all stages after the second, one finds a very large bundle of nerve fibers passing from the sinus gland toward the medulla terminalis. Some of these pass directly into the neuropile of the medulla terminalis and some ramify among the adjacent ganglion cells. In the first two adult stages the sinus gland is too closely appressed to the medulla terminalis for the presence of the nerve fibers to be readily determined. There is not, however, any evidence that there is a nerve fiber tract which extends directly to the brain as has been reported in Cambarus by Welsh (1941).

There are no obvious cell boundaries in the sinus gland. In fact there are so few nuclei to be found in any particular specimen's sinus gland that the individual cells which constitute the gland must be relatively very large. It is possible to detect canals which extend toward the blood sinus of the eye stalk; presumably these carry the secretory products to the blood stream. The secretions are in the form of large, more or less irregular, masses the amount of which varies very little regardless of the nearness or remoteness of the moulting period.

The secretions of the sinus gland give a basophilic reaction to the stains employed before, after and in the intermoult periods. In specimens fixed while in the process of moulting that portion of the sinus gland which is next to the neuropile of the adjacent optic ganglion gives an acidophilic reaction, whereas,

PLATE IV [6]

Microphotographs to show the sinus gland and X-organ in *Homarus americanus* and *Pinnotheres maculatus*. Figures 18, 19, 20, 22, 23 × 725, and figure 21 × 150; all are reduced about 25 percent. The material shown in figure 18 was fixed in Carnoy-Lebrun, that of figure 20 was fixed in Zenker-formol, and all others were fixed in Bouin-Duboscq-Brasil. The material shown in figure 22 was stained with Mallory's triple stain, and all others were stained with Foot's modification of the Masson trichrome stain.

FIGURE 18. *Homarus americanus.* A portion of the optic ganglion of an embryo fixed in the late egg stage and sectioned at five micra. The arrow indicates the characteristic secretory products of the X-organ which is surrounded by the cell layer of the optic ganglion. (Compare with Fig. 8.)

FIGURE 19. *Pinnotheres maculatus.* Section of the late egg stage embryo showing the position (arrow) of the X-organ. Sections were cut at four micra. (Compare with Fig. 1.)

FIGURE 20. *Homarus americanus.* A portion of the eye stalk of a fourth stage embryo, sectioned at seven micra, showing the close association of the X-organ with the cells underlying the eye papilla. The bulge in the exoskeleton can be noted at the top of the photograph. Note that there are fewer nuclei in the X-organ, per unit area, than in the adjacent optic ganglion. (Compare with Fig. 10.)

FIGURE 21. *Pinnotheres maculatus.* Section at eight micra of the eye stalk showing the general relationship of the various structures found therein. (Compare with Fig. 6.)

FIGURE 22. *Homarus americanus.* Section of the eye stalk of a third stage after hatching specimen (at seven micra) which shows the sinus gland lying just above the deeply staining muscle. Note that it stains much as the surrounding ganglion does and that the blood sinus is quite small.

FIGURE 23. *Pinnotheres maculatus.* Section of the sinus gland shown in figure 21 enlarged to show its structure. This is the gland of a specimen that had been starved for forty-six days. Only one nucleus is to be found in this section, and what few secretory products are seen are stained brilliantly acidophilic. Note the indefiniteness to the connective tissue framework of the gland.

[6] All photographs, excepting that of figure 20, were taken using the same equipment and materials that were used for those of Plate III. The photograph for figure 20 was made on Eastman Ortho-X film using only the X-1 Wratten filter.

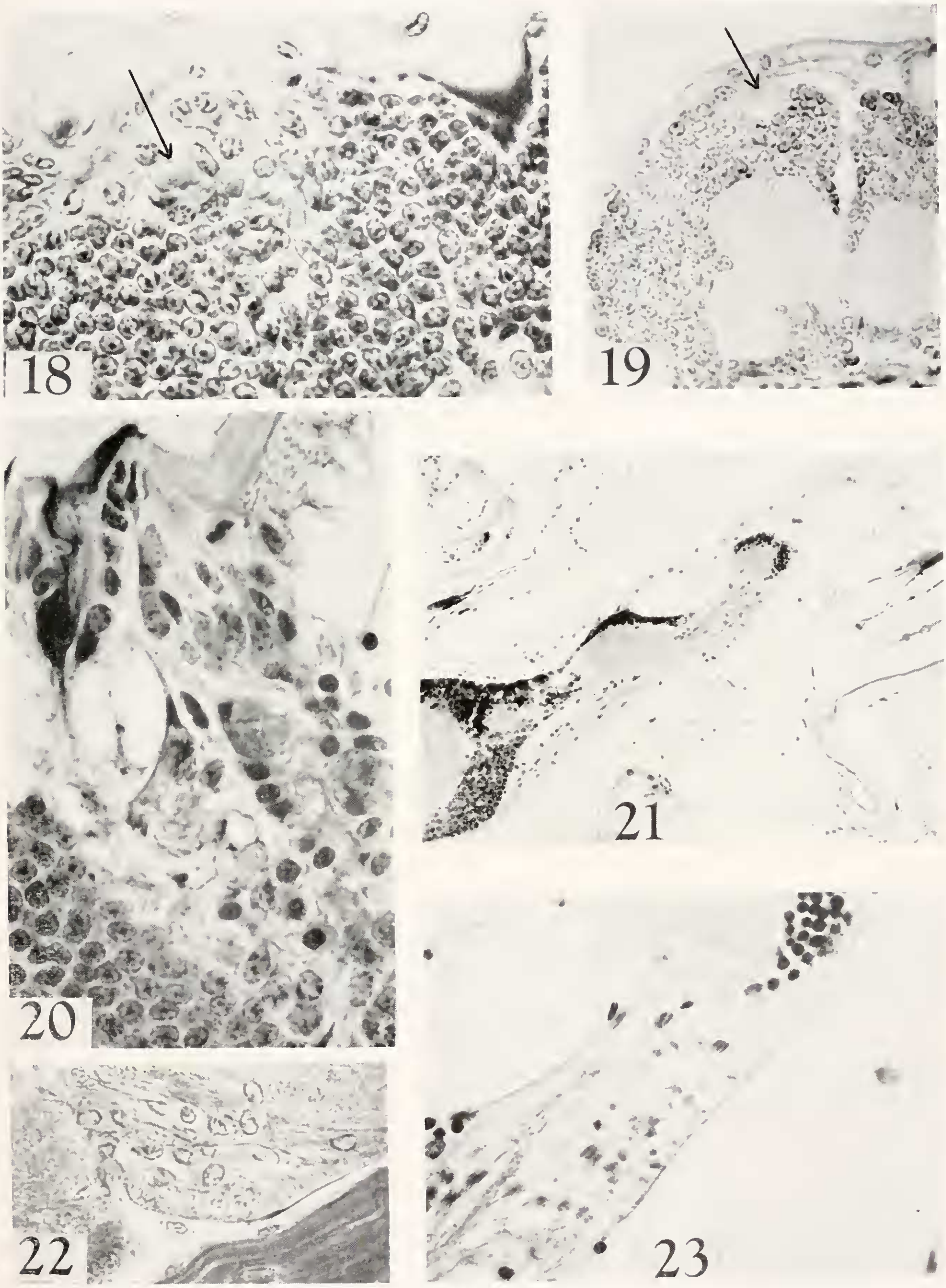

PLATE IV

the lateral portion, next to the blood sinus, gives a basophilic reaction. The acidophilic and basophilic portions blend together in the middle of the gland. Some specimens were starved for varying lengths of time. Those which had been starved for eight days exhibit both an acidophilic and basophilic reaction, but the two reactions are not regionally differentiated as is the case in specimens fixed while in the process of moulting. This reaction is found regardless of whether the specimens are fixed before, during or after the moulting period. In specimens starved for as long as 46 days (Figs. 21, 23) one finds only an acidophilic reaction regardless of the nearness or remoteness of the moulting period. Likewise, as the period of starvation is increased the amount of secretory material present in the gland is decreased although there is no evidence that the decrease due to starvation affects the frequency of moults in this particular animal.

B. *Homarus americanus*

The X-organ is found in the late egg stage (Figs. 8, 18); it is comparable in appearance to the X-organ in Pinnotheres, although there are definite structural differences in it. It is located in that part of the eye stalk that will become the median somewhat ventral side in the first stage after hatching. It is entirely surrounded by the cellular layer of the adjacent optic ganglion, but is separated from the ganglia cells by a thin connective tissue sheath. A definite bundle of nerve fibers extends from the X-organ to the medulla terminalis. The nuclei of the X-organ cells are histologically the same as those of the surrounding ganglia cells: the cytoplasm is more abundant than in the ganglia cells, and that which does not contain secretory products is clear and stains lightly. The secretory products show a series of concentric layers, when sectioned, comparable to those found in Pinnotheres; the nuclei are pushed to one side by the secretory products which nearly fill the entire cytoplasm. In all stages, under low power of the microscope, the X-organ has a similar appearance. This characteristic appearance has been described by Hanström (1939) as a "bunch of grapes." The distal portion of the X-organ extends to the median somewhat ventral portion of the hypodermis of the eye stalk. There is no evidence that there is any eye papilla formed at this time; in later stages the association of the X-organ and the eye papilla is evident. The X-organ exhibits no cyclic phenomena, cytologically, in the egg. The secretory products are always basophilic to the stains employed and vary very little in quantity.

In the first four stages after hatching (Figs. 10, 20) the X-organ increases greatly in size; this is due to the greatly increased number of cells in it and the increased amount of secretory products. Although there is a large increase in the number of cells found in the X-organ there is evidence of only an occasional mitosis after the animal has hatched. At its distal portion the X-organ comes into close contact with the exoskeleton which is bulged at this point. The cuticle of this particular region is extremely thin; this is the eye papilla (Figs. 10, 20). The eye papilla cells are found on the distal side of the X-organ between it and the ommatidia of the eye. There is no connection between the X-organ and the eye papilla as the X-organ is completely surrounded by a connective tissue sheath. No bipolar cells are found in the distal portion of the X-organ that are comparable to those described by Hanström (1937, 1939) for the adult of *Homarus* *americanus.* Cytologically there is no evidence of any cyclic phenomena in the X-organ during the first four stages after hatching; the secretory products are basophilic and the quantity is quite constant.

In the adult (Fig. 11) the X-organ no longer extends to the exoskeleton, but is found in the proximal half of the eye stalk. The basal portion of it is imbedded in the cellular layer of the distal part of the medulla terminalis. The distal portion extends well beyond the ganglionic cellular layer (to a point approximately level with the distal end of the medulla interna) into the blood sinus of the eye stalk. The X-organ occupies the same general position that it occupies in the earlier stages. In general structure it has become considerably more complex; it is now divided into a large number of units each of which is composed of from ten to twenty or more cells. Each of these units has a circular, whorled appearance. The nuclei are arranged around the periphery and the secretory products occupy the central area. A large bundle of nerve fibers passes around each of the units giving off nerve fibers to the individual cells. This arrangement gives the serial sections an appearance of being a series of whorls each of which originates from a common central stem of nerve fibers. The main bundle of nerve fibers passes between the various units and extends to the median side of the medulla terminalis. The nuclei have increased prodigiously in numbers, but still bear a marked resemblance to those of the cells of the optic ganglia. The cytoplasm of the X-organ cells is large and irregular in shape; it is filled for the most part with secretory products which have the characteristic concentric layers within them. Cells not possessing secretions have a clear lightly staining cytoplasm.

There is no evidence of cyclic phenomena associated with moulting as far as the X-organ is concerned. The basophilic reaction is found regardless of whether the eye stalk has been fixed a few days, 48 hours, six hours before, six hours, 48 hours, one, one and one-half, four or six months after moulting. Likewise, there is little change in the amount of secretory products that are evident in the X-organ of the eye stalks in the above series; the number of blue staining concretions is remarkably constant. In the case of the specimen that had not moulted for more than one year there were fewer secretory products present and more of the units contained vacuoles.

As far as can be determined the sinus gland is not formed sufficiently to be definitely recognized as such until the third stage after hatching (Figs. 9, 22). At this time it is a thin, lightly staining structure located on the dorso-lateral side of the eye stalk between the medulla interna and medulla externa. It is not very conspicuous as it does not give the typically brilliant acidophilic reaction to acid fuchsin that is found in the adult sinus gland. Structurally the sinus gland has the appearance of being a thickened portion of the neurilemma which invests the optic ganglia. The nuclei are few in number and stain precisely in the same manner as the nuclei of the neurilemma. The cell boundaries cannot be discerned; the cytoplasm seems to be confined to the connective tissue framework of the gland upon which the secretory materials are hung. The general tissue of the gland, regardless of what it is composed, stains very lightly with all the stains employed. There is a definite nerve fiber tract which extends from the sinus gland to the lateral distal border of the medulla terminalis. It is this fact that makes it possible to ascertain the presence of the sinus gland in the third stage after hatching. No such innervated structure has been found in the earlier stages.

In the fourth stage after hatching (Fig. 10) the eye stalk has increased more in thickness than in length. Consequently, the medulla interna is displaced; the sinus gland is found on the proximal portion of the medulla externa lateral to the medulla interna. This brings the sinus gland into closer proximity to the medulla terminalis. The sinus gland has increased in size with the resultant increase in the number of nuclei found in it, but the cytoplasm is still lacking the brilliant acidophilic reaction one might expect. No cell boundaries are visible; the nerve tract from the medulla terminalis is much more prominent than in the third stage after hatching.

In the adult (Fig. 11) the sinus gland occupies the same general position as in the early stages, but the eye stalk has become much more extended so that the medulla terminalis, interna and externa and lamina ganglionaris are strung out and occupy a much smaller portion of the inside of the eye stalk than they did in the early stages. As a result the sinus gland is found in the proximal half of the eye stalk on the opposite side from the X-organ. It is much more highly developed and extended than in the early stages. Situated alongside the medulla interna and extending to the proximal portions of the medulla externa it sends large finger-like processes out into the adjacent blood sinus. The nerve fiber tract extending from the sinus gland to the medulla terminalis is very large; after the protargol stain of Bodian (1937) one finds that the nerve fibers ramify among the fibers of the neuropile of the medulla terminalis and branch to all parts of the sinus gland. The framework of the gland is composed of connective tissue which stains precisely the same as the other connective tissue found in the eye stalk. There are no distinct cell boundaries observable in most preparations, but occasionally one is able to find an isolated cell which has a definite cell boundary surrounding a large irregular cytoplasmic mass. The nuclei have the same appearance as those of the early stages; they look more like connective tissue nuclei than nerve cell nuclei.

As has been pointed out above there is no striking staining reaction in the sinus gland of the third and fourth stages after hatching. In the adult, however, there are some interesting phenomena. In the series obtained for this research the following reactions are discernible: Specimens fixed several days and a few hours before moulting have the sinus gland filled with irregularily shaped secretory granules (Fig. 12) which, after Foot's modification of the Masson trichrome stain and other stains employing acid fuchsin and aniline blue, give a brilliant acidophilic reaction for the most part although there are a very few granules which react basophilically. Specimens fixed six hours, 48 hours, one and one and one-half months after moulting give three characteristic reactions. Some of the granules are brilliantly acidophilic, some are slightly acidophilic, and a number are decidedly basophilic (Fig. 13). In specimens fixed four and six months after moulting the amount of secretory material in the sinus gland is decidedly less than in those fixed during the summer months at or near the time of moulting (Fig. 14). In these cases the secretions are for the most part only slightly acidophilic with an occasional basophilic granule being found. In the specimen that had not moulted in over a year there was less secretory material in the sinus gland than was found in those (fixed in the summer months) which had moulted, but there was more than was found in those specimens fixed in the late fall and winter. The secretory material was brilliantly acidophilic, slightly acidophilic and basophilic. There was more basophilic material in this particular specimen than in any of the others. Examination of the exoskeleton showed that a new exoskeleton had been laid down underneath the old one which had not, for some reason, been shed.

C. *Cambarus virilis*

When the cyclic phenomena were found in the sinus gland of Homarus it was thought advisable to study the sinus gland of *Cambarus virilis* in which Dethier (1942) had previously reported a similar reaction. Accordingly, sections were made of the eye stalks of specimens fixed just before and just after moulting as well as of those fixed in late December. The sinus gland of specimens fixed just before moulting (Fig. 15) was filled with many irregularly-shaped granules which for the most part gave a brilliant acidophilic reaction, but there were occasional granules which were basophilic. In those specimens fixed after having completed moulting (Fig. 16) there was a sharp reduction in the number of secretion granules present; a few of these were brilliantly acidophilic, but most of them exhibited varying degrees of a basophilic reaction. In those specimens fixed late in December (Fig. 17) there were about the same number of granules as were found in the post-moult specimens, but the majority of these were acidophilic and only a few basophilic.

Discussion

Dethier (1942) in her account of the sinus gland in Cambarus states that she has been able to trace it from the first post-embryonic moult, and that it is apparently functional at that time.[7] This is not the case in the two species used for this investigation; in Homarus it has been impossible to ascertain definitely its presence until the third stage after hatching, and in Pinnotheres it could not be detected (with the techniques used) in the egg or first zoeal stages. Cytologically the evidence seems to indicate that the sinus gland in the third and fourth stages of Homarus is not a functional gland.

It has fairly well established that the color changes in Crustacea are controlled by hormones which originate in the eye stalk. As Kleinholz (1942) points out "the glandular tissue is probably the sinus gland, although the X-organ may also be concerned in this function." The apparent absence of the sinus gland in the early stages suggests that the X-organ may be functional in this capacity at this time, but the cytological evidence does not bear this out in Homarus and Pinnotheres. On the other hand, Cambarus has no X-organ which has the characteristic concretions of secretory material that are comparable to those found in Homarus and Pinnotheres. (Welsh, 1941, has found a mass of tissue on the dorso-lateral side of the medulla terminalis which he suggests may be the X-organ in *Cambarus bartoni*.)

Megušar (1912), Abramowitz and Abramowitz (1938, 1940), Brown and Cunningham (1939), Kleinholz and Bourquin (1941), and Smith (1940) have

[7] When the crayfish hatches it is a miniature adult with all appendages etc., and is comparable to a fifth or sixth stage of *Homarus americanus*.

shown that the removal of both eye stalks from crustaceans hastens the onset of moulting. Smith showed quantitatively that the removal of both eye stalks shortened the intermoult period by slightly more than 30 per cent. This probably indicates that some structure in the eye stalk, possibly the sinus gland, produces a hormone which has an inhibiting effect upon moulting. Kyer (1942) gives good evidence that the sinus gland, when active, specifically inhibits moulting and gastrolith formation. Dethier (1942) in her account of Cambarus suggests that there is an acidophilic basophilic series which is related to the period of moulting. In the cases of Homarus and Cambarus the acidophilic reaction before moulting and the basophilic one after moulting seem to indicate cyclic changes in the sinus gland which are directly related to the moulting process. Further evidence of the activity of the sinus gland is exhibited by the reduction in the amount of secretory material in it; this is most striking in Cambarus, less evident in Homarus and scarcely detectable in Pinnotheres (this is probably due to the fact that Pinnotheres passes through several moults in fairly rapid succession).

The explanation of the basophilic and acidophilic reactions in Pinnotheres is more difficult on the basis of secretory activity. If one had only the normal animals to consider it might be possible to state that the activities of the sinus gland in this species passed through a reverse acid-base reaction which were a direct result of its activity. However, in as much as the sinus gland of the starved animals, and that in the ones in the process of moulting, both give acidophilic reactions it may be that the lack of food changes the pH- of the sinus gland from a normally basic range to an acid range. Since the animal does not feed during the period of ecdysis this may account in part for the acidophilic reaction of the sinus gland at this time.

Plankmann (1935) reported that various factors (starvation, etc.) may affect the rate of moulting. The Pinnotheres that were starved for varying periods of time were kept at a temperature comparable to that of their normal environment, on a dark background and in running sea water. There was no increase in the frequency or number of moults that occurred; it was the sinus gland that showed the affect of starvation and the X-organ appeared unchanged.

In the case of retinal pigment migration Parker (1897) could find no nerve fibers supplying the distal pigment cells in Palaemonetes. This observation started the controversy of the interrelationship of the eyes and subsequently many investigations have been made upon this subject. It has been shown in a generally satisfactory manner that the sinus gland produces a retinal pigment hormone (cf. Welsh, 1941). The question is raised as to the mechanism involved in the early stages where there is no obvious sinus gland to be found. If the sinus gland is the sole controlling factor it must be assumed that the early stages are incapable of retinal pigment migration.

Further studies are necessary to give satisfactory answers to the following points which have not been completely answered in the present study:

1. From precisely what pre-existing tissue is the sinus gland formed?
2. Is the sinus gland a syncytium?
3. Is the sinus gland noncellular and merely a storage space or are there cells which periodically fill with secretory products and break down (e.g. is secretion holocrine?)?

Summary

1. The histogenesis of the sinus gland and X-organ have been studied and described for the egg, first zoea and adult stages of *Pinnotheres maculatus*.

2. The sinus gland is not found in the egg or first zoea, but it is found in all the adult stages of Pinnotheres.

3. The X-organ is found in the egg and other stages of Pinnotheres.

4. The histogenesis of the sinus gland and X-organ have been studied and described for the egg, first four stages after hatching and the adult of *Homarus americanus*.

5. The sinus gland is not found as a definitely discernible structure in Homarus until the third stage after hatching.

6. The X-organ is found in all stages of Homarus that have been studied.

7. Evidence is presented for the existence of cyclic secretion phenomena in the sinus gland of all species studied.

8. There is no evidence of the existence of cyclic secretion phenomena in the X-organ in any of the species investigated.

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